COPY OF CLAIMS DETERMINED TO BE PATENTABLE BY EPO

~~Application No. 04816631.8~~
~~University of Manitoba~~

NEW CLAIMS ~~OF 5 OCTOBER 2009 SHOWING AMENDMENTS~~

1. A method of modifying a plant phenotype, comprising:

transforming a plant with an expression vector comprising a nucleotide sequence encoding a plant non-symbiotic hemoglobin or an antisense sequence thereto, thereby yielding a transformed plant having an altered level of expression of non-symbiotic plant hemoglobin as compared to a non-transformed control plant that is not transformed to alter the level of expression of non-symbiotic plant hemoglobin,

wherein said transformed plant exhibits, under normal oxygen conditions, a plant phenotype that is modified as compared to said non-transformed control plant,

wherein said phenotype is selected from the group consisting of shoot or root apical dominance; flower color; and chlorophyll content

wherein, when said transformed plane exhibits an increased level of expression of non-symbiotic hemoglobin as compared to said non-transformed control plant, said plant exhibits increased shoot apical dominance or greater root apical dominance under normal oxygen conditions as compared to said non-transformed control plant.

2. The method of claim 1, wherein said transformed plant exhibits an increased level of expression of non-symbiotic hemoglobin as compared to said non-transformed control plant.

3. The method of claim 2, wherein said transformed plant exhibits increased shoot apical dominance under normal oxygen conditions as compared to said non-transformed control plant.

4. The method of claim 2, wherein said transformed plant exhibits reduced flower pigmentation under normal oxygen conditions as compared to said non-transformed control plant.

5. The method of claim 1, wherein said transformed plant exhibits a decreased level of expression of non-symbiotic hemoglobin as compared to said non-transformed control plant.

6. The method of claim 2, wherein said method comprises transforming said plant with an expression ~~system~~ vector comprising a nucleic acid molecule encoding a plant non-symbiotic hemoglobin.

7. The method of claim 5, wherein said method comprises transforming said plant with an expression ~~system~~ vector comprising an antisense plant non-symbiotic hemoglobin nucleic acid molecule.

~~8. A plant transformed in accordance with the method of claim 1, wherein the plant exhibits a decreased level of expression of non-symbiotic hemoglobin as compared to said non-transformed control plant.~~

~~9~~ 8. The method of claim 1, wherein said expression vector comprises an inducible promoter that permits selective induction of expression of a plant non-symbiotic hemoglobin.

~~10~~ 9. The method of claim 1, wherein said expression vector comprises a repressible promoter that permits selective repression of expression of a plant non-symbiotic hemoglobin.

COPY OF EPO OFFICE ACTIONS RELEVANT TO PATENTABILITY

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Office européen des brevets

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Application No.	Ref.	Date
04 816 631.8 - 1212	40925 EP 01	04.11.2009
Applicant The University of Manitoba		

Communication under Rule 71(3) EPC

You are informed that the Examining Division intends to grant a European patent on the basis of the above application with the text and drawings as indicated below:

In the text for the Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU MC NL PL PT RO SE SI SK TR

Description, Pages

2-4, 8, 14-24	as published
1, 5, 5a, 6, 7, 9, 11-13	received on 05.10.2009 with letter of 05.10.2009

Claims, Numbers

1-9	received on 05.10.2009 with letter of 05.10.2009

Drawings, Sheets

1/6-6/6	as published

With the following amendments to the above-mentioned documents by the examining division

Description, Pages	1,6,8,13,24**
Claims, Pages	1*

Comments

Registered Letter

* deletion of headline
** Deletion of title and amendments to bring the contents of the description in line with the granted subject matter.

A copy of the relevant documents is enclosed

The title of the invention in the three official languages of the European Patent Office, the international patent classification, the designated Contracting States, the registered name of the applicant and the bibliographic data are shown on the attached EPO Form 2056.

You are requested within a non-extendable period of **four months** of notification of this communication

1. to file 1 set of translations of the claim(s) in the two other EPO official languages;

		EUR
2a.	to pay the fee for grant including the fee for printing up to and including 35 pages; Reference 007	790.00
2b.	to pay the printing fee for the 36th and each subsequent page; number of pages: 0 Reference 008	0.00
3.	to pay the additional claim fee(s) (R. 71(6) EPC); number of claims fees payable: Reference 016	0.00
	Total amount	790.00

The mention of the grant of the patent shall be published in the European Patent Bulletin as soon as possible after the requirements concerning the translation of the claims and the payment of the fees for grant and printing, claims fees, designation fees and renewal fees as laid down in Rule 71(3), (4), (6) and (8) and (9) EPC are fulfilled.

Any divisional applications relating to this European patent application must be filed directly at the European Patent Office in Munich, The Hague or Berlin in accordance with Article 76(1) and Rule 36 EPC **before** the date on which the European Patent Bulletin mentions the grant of the patent (see Guidelines for Examination in the EPO, A-IV, 1.1.1).

If you do not approve the text intended for grant but wish to request amendments or corrections, the procedure described in Rule 71(4) EPC is to be followed.

If this communication is based upon an auxiliary request, and you reply within the time limit set that you maintain the main or a higher ranking request which is not allowable, the application will be refused (Art. 97(2) EPC).

If the enclosed claims contain amendments proposed by the Examining Division, and you reply within the time limit set that you cannot accept these amendments, refusal of the application under Article 97(2) EPC will result if agreement cannot be reached on the text for grant.

In all cases except those of the previous two paragraphs, if the fees for grant and printing or claims fees are not paid, or the translations are not filed, in due time, the European patent application will be deemed to be withdrawn (R. 71(7) EPC).

For all payments you are requested to use EPO Form 1010 or EPO Form 1010E or to refer to the relevant reference number.

After publication, the European patent specification can be downloaded free of charge from the EPO publication server https://publications.european-patent-office.org or ordered from the Vienna sub-office upon payment of a fee (OJ EPO 2005, 126).

Upon request in writing each proprietor will receive the certificate for the European patent **together with one copy** of the patent specification provided that the request is filed within the time limit of Rule 71(3) EPC. If such request has been previously filed, it has to be confirmed within the time limit of Rule 71(3) EPC. The requested copy is free of charge. If the request is filed after expiry of the Rule 71(3) EPC time limit, the certificate will be delivered without a copy of the patent specification (R.74 EPC, Decision of the President of the EPO, Special edition No.3, OJ EPO 2007, D.2).

Note on payment of renewal fees

If a renewal fee falls due between notification of the present communication and the proposed date of publication of the mention of the grant of the European patent, publication will be effected only after the renewal fee and any additional fee have been paid (R. 71(9) EPC).

Under Article 86(2) EPC, the obligation to pay renewal fees to the European Patent Office terminates with the payment of the renewal fee due in respect of the year in which the mention of the grant of the European patent is published.

Filing of translations in the Contracting States

As regards translation requirements prescribed by the Contracting States under Article 65(1) EPC, please consult the website of the European Patent Office
www.epo.org ➞Patents ➞Law ➞Legal texts ➞National law relating to the EPC
www.epo.org ➞Patents ➞Law ➞Legal texts ➞London Agreement

In case of a valid extension

As regards translation requirements prescribed by the Extension States, please consult the website of the European Patent Office
www.epo.org ➞Patents ➞Law ➞Legal texts ➞National law relating to the EPC

Failure to supply a prescribed translation in a Contracting State or an Extension State may result in the patent being deemed to be void *ab initio* in the State concerned (Article 65(3) EPC).

Important note to users of the automatic debiting procedure

The fees for grant and printing and also any additional claims fees due under Rule 71(6) EPC will be debited automatically on the date of filing of the translation of the (relevant) claims, or on the last day of the period of this communication. However, if the designation fees become due as set out in Rule 71(8) EPC and/or a renewal fee becomes due as set out in Rule 71(9) EPC, these should be paid separately by another permitted means of payment in order not to delay the publication of the mention of grant. The same applies in these circumstances to the payment of extension fees. For further details see the Arrangements for the automatic debiting procedure (AAD) and accompanying Information from the EPO concerning the automatic debiting procedure (Annexes A.1 and A.2 to the Arrangements for deposit accounts (ADA) in Supplement to OJ EPO 3/2009).

Examining Division:

Chairman:	Maddox, Andrew
2nd Examiner:	Bucka, Alexander
1st Examiner:	Holtorf, Sönke

Berger, Caroline
For the Examining Division
Tel. No.: +31 70 340 - 2363 **Branch at The Hague**

Enclosure(s): Form 2056
32 Copies of the relevant documents

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Application No.: 04 816 631.8

IV.2. Patent classification

The classification indicated on the published patent application remains unchanged. It is as follows:

INV. C12N15/82 A01H5/00 A01H3/00

IV.3. Title of the invention

The title indicated on the published patent application remains unchanged. It reads as follows:

VERFAHREN ZUM MODIFIZIEREN PFLANZLICHER PHÄNOTYPEN MIT NICHTSYMBIOTISCHEM HÄMOGLOBIN

METHOD OF MODIFYING PLANT PHENOTYPES WITH NONSYMBIOTIC HEMOGLOBIN

METHODE POUR MODIFIER DES PHENOTYPES VEGETAUX AVEC DE L'HEMOGLOBINE NON SYMBIOTIQUE

IV.4. Documentation

12.10.2009
Date

Maddox, Andrew
Chairman

Holtorf, Sönke
1st examiner

Bucka, Alexander
2nd examiner

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Application No.	Ref.	Date
04 816 631.8 - 1212	40925 EP 01	06.10.2009
Applicant The University of Manitoba		

Result of consultation

A copy of the result of consultation of 01.10.2009 is enclosed for your information.

Europäisches Patentamt · European Patent Office · Office européen des brevets

Holtorf, Sönke
For the Examining Division

Enclosure(s): Copy of result of consultation (Form 2036)

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Application No. : 04 816 631.8

Consultation by telephone with the applicant / representative

Despatch for information

Participants

Applicant:	University of Manitoba
Representative:	Mr. H.R. Andersen of Plougmann & Vingtoft
Member(s) of the Examining Division:	Holtorf, Sönke

Result of consultation

The Examiner called the applicant on the 01.10.2009 to answer a question the representative posed in the week before that date.
In his previous phone call, the representative drew the attention of the examiner to page 10, lines 3-4 which - according to the applicant - would form the basis for the amendment of claim 1 relating to the "increased root apical dominance".
In line 3-4 of page 10, the text discloses that the nsHB+ plants may exhibit "greater apical dominance in roots". According to the applicant, the terms "greater" and "increased" mean the same thing and are exchangable and the term "greater" would therefore form the basis for the term "increased".

The examiner informed the representative that the Examining Division (ED) is of the opinion that the terms "greater" and "increased" are not interchangable and if they were interchangable, the use of the term "greater" in claim 1 is advisable.

To avoid an objection according to Art. 123(2) EPC, the ED suggested to introduce the term "greater root apical dominance" into claim 1.

Application No. 04816631.8
University of Manitoba

NEW CLAIMS OF 5 OCTOBER 2009 SHOWING AMENDMENTS

1. A method of modifying a plant phenotype, comprising:

transforming a plant with an expression vector comprising a nucleotide sequence encoding a plant non-symbiotic hemoglobin or an antisense sequence thereto, thereby yielding a transformed plant having an altered level of expression of non-symbiotic plant hemoglobin as compared to a non-transformed control plant that is not transformed to alter the level of expression of non-symbiotic plant hemoglobin,

wherein said transformed plant exhibits, under normal oxygen conditions, a plant phenotype that is modified as compared to said non-transformed control plant,

wherein said phenotype is selected from the group consisting of shoot or root apical dominance; flower color; and chlorophyll content

wherein, when said transformed plane exhibits an increased level of expression of non-symbiotic hemoglobin as compared to said non-transformed control plant, said plant exhibits increased shoot apical dominance or greater root apical dominance under normal oxygen conditions as compared to said non-transformed control plant.

2. The method of claim 1, wherein said transformed plant exhibits an increased level of expression of non-symbiotic hemoglobin as compared to said non-transformed control plant.

3. The method of claim 2, wherein said transformed plant exhibits increased shoot apical dominance under normal oxygen conditions as compared to said non-transformed control plant.

4. The method of claim 2, wherein said transformed plant exhibits reduced flower pigmentation under normal oxygen conditions as compared to said non-transformed control plant.

01.10.2009
Date

Holtorf, Sönke
Examiner

5. The method of claim 1, wherein said transformed plant exhibits a decreased level of expression of non-symbiotic hemoglobin as compared to said non-transformed control plant.

6. The method of claim 2, wherein said method comprises transforming said plant with an expression ~~system~~ vector comprising a nucleic acid molecule encoding a plant non-symbiotic hemoglobin.

7. The method of claim 5, wherein said method comprises transforming said plant with an expression ~~system~~ vector comprising an antisense plant non-symbiotic hemoglobin nucleic acid molecule.

~~8. A plant transformed in accordance with the method of claim 1, wherein the plant exhibits a decreased level of expression of non-symbiotic hemoglobin as compared to said non-transformed control plant.~~

~~9~~ 8. The method of claim 1, wherein said expression vector comprises an inducible promoter that permits selective induction of expression of a plant non-symbiotic hemoglobin.

~~10~~ 9. The method of claim 1, wherein said expression vector comprises a repressible promoter that permits selective repression of expression of a plant non-symbiotic hemoglobin.

Application No. 04816631.8
University of Manitoba

NEW CLAIMS OF 5 OCTOBER 2009

1. A method of modifying a plant phenotype, comprising:

transforming a plant with an expression vector comprising a nucleotide sequence encoding a plant non-symbiotic hemoglobin or an antisense sequence thereto, thereby yielding a transformed plant having an altered level of expression of non-symbiotic plant hemoglobin as compared to a non-transformed control plant that is not transformed to alter the level of expression of non-symbiotic plant hemoglobin,

wherein said transformed plant exhibits, under normal oxygen conditions, a plant phenotype that is modified as compared to said non-transformed control plant,

wherein said phenotype is selected from the group consisting of shoot or root apical dominance; flower color; and chlorophyll content

wherein, when said transformed plane exhibits an increased level of expression of non-symbiotic hemoglobin as compared to said non-transformed control plant, said plant exhibits increased shoot apical dominance or greater root apical dominance under normal oxygen conditions as compared to said non-transformed control plant.

2. The method of claim 1, wherein said transformed plant exhibits an increased level of expression of non-symbiotic hemoglobin as compared to said non-transformed control plant.

3. The method of claim 2, wherein said transformed plant exhibits increased shoot apical dominance under normal oxygen conditions as compared to said non-transformed control plant.

4. The method of claim 2, wherein said transformed plant exhibits reduced flower pigmentation under normal oxygen conditions as compared to said non-transformed control plant.

5. The method of claim 1, wherein said transformed plant exhibits a decreased level of expression of non-symbiotic hemoglobin as compared to said non-transformed control plant.

6. The method of claim 2, wherein said method comprises transforming said plant with an expression vector comprising a nucleic acid molecule encoding a plant non-symbiotic hemoglobin.

7. The method of claim 5, wherein said method comprises transforming said plant with an expression vector comprising an antisense plant non-symbiotic hemoglobin nucleic acid molecule.

8. The method of claim 1, wherein said expression vector comprises an inducible promoter that permits selective induction of expression of a plant non-symbiotic hemoglobin.

9. The method of claim 1, wherein said expression vector comprises a repressible promoter that permits selective repression of expression of a plant non-symbiotic hemoglobin.

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Application No. : 04 816 631.8

Consultation by telephone with the applicant / representative

Despatch for information

Participants

Applicant:	University of Manitoba
Representative:	H. Rastrup Andersen of Plougman & Vingtoft a/s
Member(s) of the Examining Division:	Holtorf, Sönke

Result of consultation

On the 02.09.2009 the representative Mr. Andersen called the examiner to confirm that the email sent minutes earlier by the Examiner and which comprises a list of objections was received in good order.
The text of the email is:

"Dear Mr. Andersen,

oral proceedings (OP) have been scheduled in the above mentioned case (EP04816631) for 09.12.09
at the premises of the EPO in The Hague.

With your letter of 31.08.2009 you filed an amended set of claims 1-10 intended to overcome the

objections raised by the Examining Division (ED) in the summons to OP.

The i) deletion of subject matter relating to the term "promoter", ii) the introduction of the term expression vector, iii) the limitation to the specific phenotypes "selected from the group consisting of shoot or root apical dominace, flower colour, and chlorophyll content", and iv) the deletion of previous claims
4,7,8,12-14 is acknowledged by the ED.

However, minor objections still have to be raised:

1. The additional text at the end of current claim 1 is based on the content of original claims 7 and 8.
Original claim 7, however, only features "The method of claim 7, wherein said transformed plant exhibits increased shoot apical dominance under normal oxygen conditions as compared to said

nontransformed plant".
In contrast, current claim 1 is also directed to increased root apical dominance as a consequence of the increased level of expression of a nsHb. There is no basis for a claim directed to the increased root apical dominance. Accordingly, current claim 1 does not meet the requirements of Art. 123(2) EPC.

The term "or root" in current claim 1 should be deleted.

2. The term "expression vector" should also be introduced into current claims 6 and 7.

3. The subject matter of current claim 8 which is directed to "a plant transformed in accordance with the method of claim 1" is actually drafted as a product by process claim and therefore does not comply with Art. 84 EPC. See also Guidelines Part C, III-9, 4.12.
Even if characterized by the expression cassette it contains, the plant as such is obvious in view of document D6, as mentioned earlier under point 6 in the summons to OP. The obtained phenotype - even if not mentioned in D6 - are intrinsic properties of the plant as such.
Document D6 in Example 2 and Fig.1 discloses barley nsHb antisense constructs, page 23, second paragraph anticipates the construction of transgenic plants harbouring said constructs. An antisense construct would lead to a "decreased level of expression of nsHb" in accordance with current claim 8.
Claim 8 therefore still lacks an inventive step pursuant to Art. 56 EPC and should be deleted.

4. Due to the deficiencies mentioned above, the scheduled OP cannot be cancelled at the moment.
If cancellation of the OP is intended by the applicant, the applicant should file a letter comprising

i) an amended set of claims comprising amendments which take account of the mentioned objections,
ii) adapt the description, and
iii) withdraw the request for OP.

5. I will call you briefly to make sure you received this email in good order.

Best regards / Mit freundlichen Grüßen / Sincères salutations

Sönke Holtorf
"

Mr. Andersen wanted to contact the applicant to discuss further steps. The Examiner explained once again that for the time being the scheduled OP is maintained by the Examining Division.

02.09.2009
. . . . . .
Date

Holtorf, Sönke
. . . . . .
Examiner

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Application No.	Ref	Date
04 816 631.8 - 1212	40925 EP 01	08.09.2009
Applicant: The University of Manitoba		

Result of consultation

A copy of the result of consultation of 02.09.2009 is enclosed for your information.

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Holtorf, Sönke
For the Examining Division

Enclosure(s): Copy of result of consultation (Form 2036)

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BY FAX ONLY

Date
03-09-2009

Reference	Application No./Patent No.
40925 EP 01	04816631.8 - 1212 / 1709183
Applicant/Proprietor	
The University of Manitoba	

BRIEF COMMUNICATION

Oral Proceedings on 09.12.09

Subject: ☒ Your letter of 31.08.2009.

Communication:
- ☐ The summons to attend oral proceedings on 09.12.09 has been cancelled.
- ☐ The procedure will be continued in writing.
- ☒ The date fixed for oral proceedings is maintained.
- ☐ A new date will be set later.
- ☒ Pls acknowledge receipt of this fax by return fax.
- X The minutes of the telephone consultation are sent by separate mail.

Please take note.

For the Examining Division

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For any questions about this communication:
Tel.:+31 (0)70 340 45 00

Date
09-07-2009

Reference	Application No./Patent No.
40925 EP 01	04816631.8 - 1212 / 1709183
Applicant/Proprietor	
The University of Manitoba	

Summons to attend oral proceedings pursuant to Rule 115(1) EPC

You are hereby summoned to attend oral proceedings arranged in connection with the above-mentioned European patent application.

The matters to be discussed are set out in the communication accompanying this summons (EPO Form 2906).

The oral proceedings, which will not be public, will take place before the Examining Division.

on 09.12.09 at 10.00 hrs, EPO Rijswijk
Patentlaan 2, NL-2288 EE Rijswijk (ZH)

No changes to the date of the oral proceedings can be made, except on serious grounds (see OJ EPO 1/2009, 58). If you do not appear as summoned, the oral proceedings may continue without you (R. 115(2) EPC, see also OJ EPO 10/2008, 471).

Your attention is drawn to Rule 4 EPC, regarding the language of the oral proceedings, and to the Special edition No. 3 OJ EPO 2007, L.1., concerning the filing of authorisations for company employees and lawyers acting as representatives before the EPO.

The final date for making written submissions and/or amendments (R. 116 EPC) is 09.11.09.

The actual room number as well as the waiting room numbers will be given to you by the porter in the foyer at the above EPO address.

Parking is available free of charge in the underground car park (see map enclosed).

1st Examiner:
Holtorf S

2nd Examiner:
Bucka A

Chairman:
Maddox A

For the Examining Division

Europäisches Patentamt · European Patent Office · Office européen des brevets

Claudepierre, Mireille

Annexes:
Confirmation of receipt (Form 2936)
Communication (EPO Form 2906)

The examination is being carried out on the **following application documents**:

Description, Pages

1-24 as published

Claims, Numbers

1-16 received on 25.05.2009 with letter of 22.05.2009

Drawings, Sheets

1/6-6/6 as published

1. The following observations and comments relate to the communications dated 04.09.2008 (C1), 20.01.2009 (C2) and the respective letters of reply of the applicant dated 19.12.2008 (L1) and 22.05.2009 (L2).

2. The arguments put forward by the applicant in his letter of reply (L2) were carefully considered by the Examining Division (ED).

3. The following items will form the basis for the discussion in the Oral Proceedings:

4. The amendments filed with letter of 22.05.2009 (L2) do not meet the requirements of Art. 123(2) EPC and do extend beyond the content of the application as originally filed.

Current claim 1 is referring to a method wherein the expression system is characterized to comprise a "promoter thereof", i.e. of the plant non-symbiotic hemoglobin (nsHb) as mentioned in the first part of claim 1.
There is no basis for such an amendment, no basis could be identified for an expression vector or expression cassette which would solely comprise a plant non-

symbiotic hemoglobin-specific promoter region and which would lead to a plant with a modified phenotype.

5. **Novelty** (Art. 54 EPC)
Many of the phenotypes as defined in current claim 1 are still directed to phenotypes which have been broadly defined; e.g. "leaf size", "leaf length".
Broad interpretation of some of these phenotypes renders prior art methods to fall within the scope of claim 1. Document D1 (Hunt et al., PNAS, 2002) discloses the overexpression of an Arabidopsis-specific and a Parasponia-specific nsHb in transgenic A. thaliana plants, the constitutive expression of which leads to tolerance towards hypoxia. The generated plants also show vigorous growth **under nonhypoxic conditions** and are 50% larger in weight than the controls at 14 days. Reduction in the number of root hairs and increased number of lateral roots is also observed. Young plants have faster growth of shoots and roots. See Fig. 2A and page 17198, right column, last paragraph.
The plant nsHb-expressing transgenic plants of Fig. 2A - grown under normal oxygen conditions after having been subjected to hypoxic stress - show e.g. increased leaf sizes/lengths.

Accordingly, the subject matter of claims 1,2,9 lacks novelty over D1 pursuant to Art. 54 EPC.

6. **Inventive Step** (Art. 56 EPC)
6.1 Documents D5 and D6 disclose the recombinant expression of the sense and antisense constructs of the barley nsHb of the current application in transgenic alfalfa roots and maize cells. Whereas D6 only teaches the generation of cell cultures, D6 states on page 23, second paragraph, that "plants containing the Hb expression vector described above engineered for expression in a given plant will have improved agronomic properties, such as, for example, germination, seedling vigour, reduced cellular levels of fermentation products, increased oxygen uptake, and increased tolerance to hypoxic conditions".
The mere regeneration of plants from the transgenic plant cells of D6 is obvious for the skilled artisan.
The subject matter of current claim 11 is lacking an inventive step according to Art. 56

EPC.

6.2 Moreover, the objection made under point 5 of C1 (Art. 56 EPC) is maintained for current claims 1-11,15,16 by the Examining Division.
It further appears that the identified problem has not been solved for the subject matter of current claims 12-14. The current application is silent about any proven effect on any of the plant hormones listed in claim 13.
The later published documents D10-D12 only speculate about the function of the nsHBs in NO scavenging (see D10). No data are provided that would indicate any relevant modification of the plant's hormone balance mediated by modulating the NO concentration.
Document D10 is teaching on page 922 that "it has been shown that NO modulates growth by a so far unknown mechanism".

7. **Clarity** (Art. 84 EPC)
Current claim 1 is still referring to an "expression system". An objection pursuant to Art. 84 EPC was already made under point 3.4 of D2. The term should be exchanged for either "expression cassette" or "expression vector". A basis is provided on page 8, line 2 of the current application.

8. **Oral Proceedings**
The present set of claims does not meet the requirements of the EPC and refusal of the application is therefore to be expected pursuant to Art. 97(2) EPC. In view of your request under the demands of Article 116 EPC, the examining division hereby invites you for Oral Proceedings for the date and time indicated in this communication, to be held within the premises of the EPO in The Hague.

The deficiencies with respect to the EPC are outlines above and will form the basis of the proceedings.

7.3 The attention of the applicant is drawn to the fact that further observations and documents for the preparation of the Oral Proceedings can only be filed at the latest one month before the stated date of the Oral Proceedings and said documents have to fulfill the requirements of Art. 123(2) EPC.

Facts or evidence provided after this date could be disregarded by the examining division (Rule 116(1) EPC; Guidelines E-III, 8.6).

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Application No.: 04 816 631.8

Preparation for oral proceedings - Instructions to Support Service

Oral proceedings are to be held in connection with the above patent application

1. The matters to be discussed are set out in the annex (Form 2906)

2. Dispatch the summons using Form 2008/2310 and Form 2906 for the parties to attend on:

Day 09.12.2009 Time 10:00

ROOMS

Room booked

ORAL 01, 02, 03 and 05 coded

..... Date Initials

2.1 Parties' submissions in preparation for the oral proceedings, if any, should be made no later than

1 month(s)

before the date of the oral proceedings (transfer to Form 2008.1 / 2310.1)

2.2 Encode ORAL(04) coded

..... Date Initials

2.3 Dispatch Form 2008.7 / 2310.7 to division

..... Date Initials

3. ☐ Arrange for the following special equipment to be provided in the conference room:

..... Date Initials

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4. Request language service to provide simultaneous interpretation facilities as necessary

..... Date ..... Initials

5. Return the dossier to primary examiner after dispatching the summons

..... Date ..... Initials

6. Check that summons has been received (Form 2936 / advice of delivery)

7. 15 days before the oral proceedings:
- dispatch the dossier to the primary examiner.

19.06.2009
Date

Maddox, Andrew
Chairman

Bucka, Alexander
2nd examiner

Holtorf, Sönke
1st examiner

.....
Legal member

The examination is being carried out on the **following application documents**:

Description, Pages

1-24 as published

Claims, Numbers

1-16 received on 19.12.2008 with letter of 19.12.2008

Drawings, Sheets

1/6-6/6 as published

1.1 The newly filed claims 1-16 are allowable in view of Article 123(2) EPC as the subject matter does not extend beyond the content of the application as filed.

1.2 As indicated by the applicant, the previous communication indeed was directed to the subject matter of claims 1-21 as amended under PCT Art. 34. Page 1 of the examination report erroneously stated that claims 1-23 as published were the basis of the examination.

2.1 The deletion of claims 2-6,9,11,18, and 19 (published set of claims 1-23) is acknowledged by the Examining Division (ED).

2.2 The new claims 15 and 16 appear to be allowable pursuant to Art. 123(2) EPC. However, although limiting the subject matter of current claim 1 to methods of introducing an expression cassette into said plants, said claims 15 and 16 are still directed to any transgene which would have a direct or indirect effect on the induction or repression of expression of the plant's endogenous non-symbionic hemoglobins. Accordingly, the objection raised under item 4.1 of the previous communication still applies for claims 15 and 16.

3.1 Current claim 1 is still relating to any method of "transforming a plant to alter the level of expression of non-symbiontic plant hemoglobins" in a plant, rendering said subject matter too broad. The objection was already raised under item 4.1 of the

previous communication and still applies. The objection made under point 2.2 of the current communication also applies for the broadly drafted methods of current claims 1 and 12-14.

3.2 The incorporation of parts of previous claim 2 into current claim 1 still leaves the reader in doubt about the relative broad terms like e.g. "plant shape", "flower colour", which describe the alteration of the plant's phenotype. The determination of the flower colour of a plant is also depending on the subjective view of the observing person. Many of the parameters depend on the time of measuring of the given plant and vary depending on the developmental stage of said plant. Claim 1 only compares a "transformed" against a "non-transformed" plant.

3.3 Depending claims 2-8 relate to the ill-defined "transformed plant" of current claim 1 and also lack clarity pursuant to Art. 84 EPC.

3.4 Likewise, the "expression system" of current claims 9 and 10 is not stringently defined.

4. In the absence of any stringent definition of the plant of current claim 11 based on the introduced expression construct, the objection raised under item 4.3 of the previous communication still applies for current claim 11.

5. The arguments as presented by the applicant in his letter of reply and relating to the objection concerning the lack of an inventive step of previous claims 1-21 were carefully studied by the ED. The objection raised under point 5 (Art. 56 EPC) of the previous communication is, however, still maintained by the ED.

6. Conclusion

In accordance with Rule 137(3) EPC the Examining Division may decide not to accept further amendments if they do not intend to overcome the raised objections.
In the latter case, refusal according to Art. 97(2) in conjunction with Rule 137 (3) EPC is to be expected.

Should the Applicant insist in maintaining the present set of claims, refusal of the application under Article 97(2) EPC is to be expected, for the reasons outlined above and in previous communications. In the latter case, Oral Proceedings, as requested by

the Applicant, will be appointed.

Sönke Holtorf

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Application No	Ref.	Date
04 816 631.8 - 1212	40925 EP 01	20.01.2009
Applicant The University of Manitoba		

Communication pursuant to Article 94(3) EPC

The examination of the above-identified application has revealed that it does not meet the requirements of the European Patent Convention for the reasons enclosed herewith. If the deficiencies indicated are not rectified the application may be refused pursuant to Article 97(2) EPC.

You are invited to file your observations and insofar as the deficiencies are such as to be rectifiable, to correct the indicated deficiencies within a period

of 4 months

from the notification of this communication, this period being computed in accordance with Rules 126(2) and 131(2) and (4) EPC. One set of amendments to the description, claims and drawings is to be filed within the said period on separate sheets (R. 50(1) EPC).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Art. 94(4) EPC).

Holtorf, Sönke
Primary Examiner
For the Examining Division

Enclosure(s): 3 page/s reasons (Form 2906)

Registered Letter

The examination is being carried out on the **following application documents**:

Description, Pages

1-24 as published

Claims, Numbers

1-23 as published

Drawings, Sheets

1/6-6/6 as published

1. The following documents are considered relevant for the current application:

D1: Hunt. P.W., et al.; PNAS, (2002)
D2: US6372961
D3: WO9812913
D4: WO2004/087755
D5: Igamberdiev, A.U., et al.; Planta (2004)
D6: WO0000597
D7: Holmberg, N., et al.; Nature Biotechnology (1997)
D8: Seregélyes, C., et al.; Plant Science (2003)

2. The following documents (D9-D12) are cited by the examiner (see the Guidelines, C VI-24, 8.4). A copy of each of the documents is annexed to the communication and the numbering will be adhered to in the rest of the procedure:

D9: Heckmann, A.B., et al., PMB (2006), Vol. 61: pp. 769-779
D10: Hebelstrup, K.H., and E.O.Jensen, Planta (2008), Vol. 227: pp. 917-927

D11: He, Y., et al., Science (2004), 305: pp. 1968-1971
D12: Hebelstrup, K.H., et al.; Physiologia Plantarum (2006), 127: pp. 157-166

3.1 The current application is dealing with the modification of a plant's phenotype by altering the expression level of "non-symbiontic" hemoglobin (nsHb) in said plants.

Symbiontic hemoglobins (leghemoglobins) originate from plants that are capable of participating in a microbial symbiosis; said hemoglobins are functioning in the regulation of oxygen transport to the symbiontic nitrogen fixing bacteria.
The recently discovered non-symbiontic hemoglobins (nsHb) appear to represent evolutionary predecessors of the leghemoglobins and are widespread in the plant kingdom.
The "non-symbiontic" hemoglobins (nsHb) are subdivided into class 1, class 2, and class 3 (truncated Hbs).

The Examples section of the current application teaches the construction of sense (Hb+) and antisense (Hb-) expression constructs comprising the barley-specific nsHb transgene.
The Figures depict transformed alfalfa plants.
Various morphological, growth- and yield-related parameters were studied and analyzed in the obtained transgenic plants:

The Hb+ transgenic alfalfa plants exhibited:
- elevated total chlorophyll content
- 32-11% more shoot yield in early vegetative growth phase
- increased root yield
- increased shoot:root ratio
- lower leaf:stem ratio
- increased yield per shoot
- increased stem weight

- sooner flowering
- mean internode length increased
- mean area per leaflet increased
- thicker stems
- elevated stem weights
- elongated and needled leaflets with longer petioles
- late rooting of stem cuttings
- thicker taproots
- fewer lateral roots

The Hb- transgenic alfalfa plants exhibited:
- intensity of flower colour increased as nsHb expression declined.
- diminished total chlorophyll content
- elevated leaf:stem ratio
- mean internode length decreased (impaired stem elongation and leaflet expansion)
- mean area per leaflet decreased (impaired stem elongation and leaflet expansion)
- compressed oval leaflets, shortened petioles
- more adventitious rooting of stem cuttings
- thin taproots
- apical dominance declines

Overexpressors also exhibit increased uptake or metabolism of nutrients whereas plants that have a downregulated nsHb-expression exhibit a reduced uptake and reduced metabolism of nutrients as compared to non-transformed plants.
The effect of nsHbs on the uptake and metabolism of iron (Fe) is reversed.

To summarize, nsHb+ plants appear to be characterized by an accelerated morphological development relative to control and nsHb- plants.
All parameters, however, seem to depend heavily on the time of their measuring, i.e. on the harvest dates (DATP).

The current application claims a newly discovered effect of plant-specific nsHbs on the plant's phenotype and it's mineral nutrition (modifying the plant's uptake, concentration and

metabolism of nutrients) under normal oxygen growth conditions when transforming said plants with sense or antisense constructs harbouring said plant-specific nsHbs.
Apart form an effect on various plant phenotypes, the applicants also claim an effect on the modification of the plant's response to plant hormones.

The mechanisms is suggested to relate to changes in NO levels which in turn affect hormone expression and the plant's phenotypic response. Plants overexpressing nsHbs will decrease the effect of hormones that have NO as a signal transduction component, while plants with a downregulated expression of nsHbs will exhibit an increase of the effect of said hormones on the plant.

3.2 Later published evidence (see D10-D12) gives a clearer picture about the function and role of the nsHbs in plants.

The function of nshemoglobins in NO scavenging during oxygen deficiency stress conditions was shown in the prior art (see D5).
The toxic effect of the application of 300uM SNP was reduced through the overexpression of alfalfa-specific nsHb in transgenic tobacco plants. see D8.

It appears that plant non-symbiontic hemoglobins serve in NO scavenging (see D10); apart from the role in hypoxic stress, class 1 and class 2 nshemoglobins also play a general role under non-stressed conditions where they are essential for normal development by controlling the level of NO. See D12.

The metabolite NO has been shown to be a growth stimulating signal in pants and has a role in meristem identity and floral transition (see D11, He et al. 2004). NO stimulates growth at low concentration (= high NO scavenging activity in nsHb-overexpressing plants) and is growth inhibitory at high concentrations (=low NO scavenging activity in nsHb-downregulated plants).

4. Clarity and Novelty (Art. 84 and 54 EPC)
4.1 Current claim 1 is relating to a method of modifying a not further defined "plant phenotype" by altering the level of nsHb expression in a plant.
The mentioned phenotypes in the two last lines of claim 1 are only conditional.

Many other mentioned phenotypes are broadly defined (e.g. "shoot or root apical dominance" - not clear in which direction said apical dominance is influenced; plant shape, early versus late flowering, metabolism of nutrients, "altered response" to a plant hormone of claim 19, to name but a few) and leave too much room for interpretation which agronomic phenotypes would fall within the scope of the respective claims.
In fact, many observed phenotypes of the prior art would - when strictly interpreting the wording of the current claims - fall within the scope of claims 1-21 and the corresponding documents would in fact be novelty-destroying for said claims pursuant to Art. 54 EPC.

The wording "transforming a plant to **alter the level of expression** of non-symbiontic plant hemoglobins in the plant" of current claim 1 is not limited to the introduction of sense or antisense constructs into said plants using techniques of genetic engineering, but is instead directed to any method of "transforming a plant" that would have a direct or indirect effect on the expression of a plant's nsHb.
Such formulation also encompasses the introduction of any other transgene having a direct or indirect effect on the expression of a plant's nsHb whereas the current application only provides experimental evidence for the recombinant expression of sense or antisense constructs of the barley nsHb clone.

4.2 The term "non-symbiontic" plant hemoglobin is misleading and creates ambiguity.
Document D9 is teaching that plant class 1 hemoglobins are known which originate from nitrogen-fixing actinorhizal plant Myrica gale and which share high identity to non-symbiontic hemoglobins. Their expression pattern, however, is also equivalent to symbiontic hemoglobins pointing to symbiontic as well as non-symbiontic specificities, i.e. a bi-functional role similar to the hemoglobin gene from Parasponia (compare D1 and D9, page 770).
It therefore appears that plant hemoglobins with non-symbiontic function also appear in plants which are capable of participating in microbial symbiosis.

As currently drafted, the Parasponia-specific hemoglobins as disclosed in D1 and the Myrica-specific hemoglobins as disclosed in D9 would fall within the term "non-symbiontic" hemoglobin as used in the current specification.

It is not at present apparent how the plant-specific hemoglobins as used in the current application can be characterized and distinguished from the symbiontic or symbiontic/non-

symbiontic Hbs (see D9 and D1).

4.3 The plants of current claims 16-18 are defined by being "transformed in accordance with the method of claim 1".
Apart from being defined by the process of obtinaing them ("product-by-process" claim, Art. 84 EPC), such plants recombinantly overexpressing a plant-specific nsHb have already been disclosed in the prior art. See D1, D4 and D8.
Even if not all effects on the plant's agronomic parameters, the plant's phenotype and development have been thoroughly determined in D1, D4 and D8; the modifications originating from the overexpression and downregulation of the nsHbs in said plants are intrinsic properties of said plants.
Accordingly, the subject matter of current claims 16-18 is lacking novelty over the prior art pursuant to Art. 54 EPC.

5. Inventive Step (Art. 56 EPC)
5.1 Document D1 (Hunt et al., PNAS, 2002) is the closest prior art and discloses the overexpression of an Arabidopsis-specific and a Parasponia-specific nsHb in transgenic A. thaliana plants, the constitutive expression leads to tolerance towards hypoxia. The generated plants also show vigorous growth under nonhypoxic conditions and are 50% larger in weight than the controls at 14 days. Reduction in the number of root hairs and increased number of lateral roots is also observed. Young plants have faster growth of shoots and roots.
The difference to the current application is the use of an nsHb from another plant species and a thorough analysis of all phenotypic effects at different developmental stages.
The problem is the provision of a method to modify other plant-specific phenotypes through the use of alternative plant-specific nshemoglobins.
The solution is the use of the barley-specific nsHb transgene to modify a multitude of phenotypes in transgenic plants.

5.2 The prior art already teaches the effect of the overexpression of plant-specific nsHbs on the phenotype of the transgenic plant and on many agronomically interesting

parameters, see D1.
Moreover, document D8 (Seregélyes et al., Plant Science, 2003) is teaching the role of nsHb in NO metabolism. The alafalfa-specific Mhb1-cDNA was constitutively overexpressed in transgenic tobacco plants. Germination of seeds was less retarded under NO treatment. The results support the conclusion that non-symbiontic hemoglobins play a role in NO-dependent physiological responses. The less-retarded seedlings show a modified phenotype in the sense of many of the generally drafted current claims.
An effect of the expression of plant-specific nsHbs on the plant's phenotype in general is further suggested in Document 2 (US6372961) and D4 (2004/087755). D3 discloses the heterologous expression of maize-specific hemoglobin genes in plants for manipulating seed germination, seedling and overall growth, and the metabolism of said plants.
D4 teaches methods for improving growth (e.g. increased biomass) and altering growth characteristics to positively affect crop yield in plants by modifying the expression of the plant class 2 non-symbiontic hemoglobin levels.
The modification of the plant's architecture is disclosed, see page 7, third paragraph.
The nsHb is the Beta vulgaris class-2 hemoglobin gene.
Transgenic A. thaliana plants were generated expressing the A. thaliana-specific AtHb2 gene, the resulting plants show increased growth under salt stress conditions, see Table 2.

Moreover, Document D5 (Igamberdiev et al., Planta, 2004) and D6 (WO0000597) are disclosing the successful recombinant expression of the transgene used in the current application, namely the barley sense and antisense class 1 hemoglobin-constructs (Hb+ and Hb-) in alfalfa root cultures and maize cell culture, respectively.

Document D3 (WO9812913) teaches the recombinant expression of a Chromobacterium-specific hemoglobin (Vitreoscilla Vhb gene) in transgenic tobacco plants leading to quicker germination, faster growth, higher crop yields, earlier flowering and higher levels of metabolites (see page 6, lines 19-27 and (p. 20, line 29 to page 21, line 2, page 22, lines 1-2, Table 1). D3 also describes how potential transgenic plants are screened for desired phenotypes, see page 16, line 9 to page 17, line 2. Some parameters (e.g growth rate, height increase, vegetative yield, germination rate, chlorophyll content) to be examined during screening of said transgenic plants are suggested to evaluate which agronomic traits have been modified by the overexpression of the hemoglobin gene. See also page

19, line 27 and page 21.

The use of plant-specific nsHbs to affect the plant's phenotype and agronomic parameters was already disclosed in the prior art. The prior art also suggests to screen transgenic plants expressinh nsHbs for other desired agronomic parameters and also discloses how to achieve this goal (D3).

Faced with the identified problem of the current application, the skilled artisan would be aquainted with the presence of the barley sense and antisense class 1 hemoglobin-constructs and with the potential effect of nsHbs on other interesting traits of a transgenic plant. Using the suggested screening methods of D3, the skilled artisan would undoubtedly arrive at the methods and plants of current claims 1-21.

5.3 The subject matter of claims 1-21 is hence obvious and does not involve an inventive step pursuant to Art. 56 EPC.

6. Conclusions

The applicant is requested to file new claims which take acount of the above comments.

It is not at present apparent which part of the application could serve as a basis for a new, allowable claim. Should the applicant nevertheless regard some particular matter as patentable, an independent claim including such matter should be filed taking account of Rule 43 EPC. The applicant should also indicate in the letter of reply the difference of the subject-matter of the new claim vis-à-vis the state of the art and the significance thereof.

In order to facilitate the examination of the conformity of the amended application with the requirements of Article 123(2) EPC, the applicant is requested to clearly identify the amendments carried out, irrespective of whether they concern amendments by addition, replacement or deletion, and to indicate the passages of the application as filed on which these amendments are based. If the applicant regards it as appropriate these indications could be submitted in handwritten form on a copy of the relevant parts of the application as filed.

Sönke Holtorf

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Application No.	Ref	Date
04 816 631.8 - 1212	40925 EP 01	04.09.2008
Applicant: The University of Manitoba		

Communication pursuant to Article 94(3) EPC

The examination of the above-identified application has revealed that it does not meet the requirements of the European Patent Convention for the reasons enclosed herewith. If the deficiencies indicated are not rectified the application may be refused pursuant to Article 97(2) EPC.

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of 4 months

from the notification of this communication, this period being computed in accordance with Rules 126(2) and 131(2) and (4) EPC. One set of amendments to the description, claims and drawings is to be filed within the said period on separate sheets (R. 50(1) EPC).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Art. 94(4) EPC).

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Holtorf, Sönke
Primary Examiner
For the Examining Division

Enclosure(s): 9 page/s reasons (Form 2906)
XP008095926; He,Y., Science,305:1968
Hebelstrup,Planta,227:917 ; Heckmann,PMB,61:769

Registered Letter